THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 63: 356–361, 1974

Vol. 63, No. 4
Printed in U.S.A.

INHIBITION OF PROSTAGLANDIN SYNTHESIS AND HUMAN EPIDERMAL ENZYMES BY AUROTHIOMALATE IN VITRO: POSSIBLE ACTIONS OF GOLD IN PEMPHIGUS*

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ABSTRACT

Concentrations of gold in skin achieved during systemic chrysotherapy inhibited prostaglandin synthesis in vitro, using a sheep vesicular gland dioxygenase system. Two human epidermal enzymes, acid phosphatase and tryptophanyl-tRNA synthetase, were also inhibited by gold in vitro, but at higher concentrations; the latter action was blocked by 2-mercaptoethanol. Antiepithelial antibody titer, and immunoglobulin synthesis as determined by serial herpes simplex complement-fixing antibody titer, were unaffected by gold. Furthermore, gold concentrations in skin did not correlate with the clinical activity of pemphigus. These findings suggest some possible mechanisms of gold action in pemphigus, such as interruption of the inflammatory cycle and inhibition of skin enzymes involved in blister formation.

Chrysotherapy is beneficial in the management of some patients with pemphigus [1] and rheumatoid arthritis [2–4] although its mechanism(s) of action has not been fully elucidated. An understanding of the site and mode of action of gold in pemphigus might result in an improved treatment schedule and the application of chrysotherapy to other dermatologic conditions. Accordingly, an experimental protocol was designed to determine the effects of gold on a number of biologic systems.

Several hypotheses have been advanced which may explain the action of gold in pemphigus and rheumatoid arthritis [5–7]. Likely possibilities include: (1) interruption of the inflammatory cycle by inhibition of prostaglandin synthesis; (2) inhibition of skin enzymes responsible for blister formation; (3) interference with antigen–antibody complex formation; and (4) immunosuppression. To test these considerations, we studied the effects of gold sodium thiomalate on: (1) sheep vesicular gland dioxygenase, an enzyme that functions in the sequence of prostaglandin synthesis; (2) several sulfhydryl-containing enzymes, human epidermal acid phosphatase, and tryptophanyl-tRNA synthetase [8]; (3) the antiepithelial antibody immunofluorescent test; and (4) the synthesis of immunoglobulins, by comparing serum herpes simplex complement-fixing antibody titers before and during chrysotherapy. Skin gold levels were also measured to determine the relationship of the clinical response of pemphigus to tissue gold concentrations.

EXPERIMENTAL PROCEDURES

Effect of gold on prostaglandin synthesis

Sheep vesicular gland dioxygenase was assayed as described by Smith et al [9,10]. Gold sodium thiomalate‡ (GTM) in final concentrations of 0–66 μM was present in the reaction mixture. Dioxygenase was prepared by homogenizing 50 mg of acetone powder of sheep vesicular gland (Upjohn) in 1 ml of 0.1 M Tris–HCl (pH 8.5) with a Dounce ball-type homogenizer. Dioxygenase [9] was activated by adding sufficient 0.1 M phenol to the enzyme preparation so that the final concentration of phenol was 0.67 M. The mixture was maintained at room temperature for a minimum of 30 min prior to testing.

Enzyme preparations and GTM were placed in the side of the electrode holder of an oxygen monitor and incubated for 0–7 min. Immediately thereafter, arachidonic acid (Lipid Organic Research) (20 μM final concentration) was added to the mixture and the reaction rate (μM/min) determined by continuous measurements of oxygen uptake, using an oxygen electrode. Oxygen absorption was measured with a Yellow Springs Instrument Company oxygen monitor Model 53 equipped with a Model 5301 bath assembly and attached to a Beckman Model 100502 Linear-Log-Ten-Inch Potentiometric Recorder. Readings were made using the percent transmittance setting of the recorder. The oxidation of arachidonic acid (20:4, n-6) by the phenol-activated vesicular gland dioxygenase was determined by measuring oxygen absorption at a constant temperature of 30° ± 0.5°C. At the termination of each experiment (in the presence of GTM) soy bean lipoxygenase (Sigma) was added to the reaction mixture and the additional consumption of oxygen recorded [9]. For purposes of control, the buffer, salicylates, and indomethacin were similarly tested. To verify that oxygen uptake was directly related to prosta-

This investigation was supported in part by USPHS Grant AM 14941-01 and a research grant from Hoffmann-LaRoche, Inc., Nutley, New Jersey.

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† Dr. Penneys is a recipient of a fellowship from the Society for Investigative Dermatology.

‡ Gold sodium thiomalate was obtained commercially as Myochrysine from Merck, Sharp & Dohme.

glandin synthesis, [1-^{14}C]arachidonic acid (Applied Science Lab.) was incubated as described above. The solution was extracted by Folch mixture and subjected to the thin-layer chromatography as described by Smith and Lands [9].

Effect of gold on skin acid phosphatase and tryptophanyl-tRNA synthetase activity

Acid phosphatase was studied as a representative lysosomal hydrolase that may function in blister formation [11]. Tryptophanyl-tRNA synthetase was studied because it is a human epidermal sulfhydryl enzyme that is available in near homogeneous form which allowed for direct analysis of kinetic experiments.

Split-thickness skin specimens were removed from normal human volunteers with a Davol dermatome. Whole skin was homogenized with a glass-glass homogenizer in 10 mM citrate buffer, pH 5.0, at 2°C, containing 0.1% Triton X-100. The homogenate was centrifuged at 10,000 × *g* for 30 min.

Acid phosphatase activity in the supernatant fraction was determined by the method of Ohkawara et al [12] using p-nitrophenyl phosphate as substrate. Dose-related inhibition experiments were performed using increasing GTM concentrations (ranging from 10–1000 μg/ml), and similar studies were done employing sodium thiomalate as a control.

Human epidermal tryptophanyl-tRNA synthetase (850-fold purified) was assayed as previously described [13]. The variability of the assay technique is ±5%. This enzyme catalyses a two-step reaction involving the activation of tryptophan to form enzyme-bound tryptophanyl adenylate with the release of pyrophosphate, and the transfer of activated tryptophan to $tRNA^{trp}$ to form Trp-tRNA. L[-3-^{14}C]-tryptophan (New England Nuclear Corporation) was purified as described previously [14] with spectrophotometric determination of its concentration [15]. Brewers' yeast tRNA (Schwarz Bioresearch) contained 38 pmoles $tRNA^{trp}$ per A_{260} unit.§ Inhibition experiments with concentrations of gold ranging from 1–100 μg/ml were performed. The effects of 20 and 50 mM 2-mercaptoethanol (Kodak) on this system were investigated using a concentration of GTM that produced 50% inhibition of enzyme activity in the absence of added thiol. Kinetic constants and patterns, using a 2-min assay as previously described [16], were determined using an IBM 370 computer with the program of Hanson et al [17] based on the theory of Bliss and James [18].

Effect of gold on the antiepithelial antibody (AEA) immunofluorescent test

Sera obtained from 5 patients with pemphigus were frozen at −20° until tested. AEA titers were determined as previously described with human skin, monkey esophagus, or guinea-pig lip as substrate [19].

Effect of gold on antiepithelial antibody titer. AEA-positive pemphigus sera were diluted with: GTM; sodium thiomalate (TM), prepared by treating thiomalic acid (Aldrich) with 1 M NaOH to a final pH of 6.0; or, 10 mM potassium phosphate buffer, pH 7.2; and incubated at room temperature for 30 min. Final concentrations of GTM were 1 and 5 mg/ml, and 1, 5, 10, 50, 100, and 500 μg/ml. Concentrations of TM were one-half those used with GTM since GTM is approximately 50% gold by weight. Dilutions were made with the above-named buffer. AEA titers of gold-treated, TM-treated, and buffer-treated sera were compared.

Effect of gold on epidermal antigen. Monkey esophagus tissue was incubated with GTM (5 mg/ml), TM (2.5 mg/ml), or phosphate buffer for 30 min at 37°C. These tissues were then used as substrates to determine AEA titers on sera of known positivity.

Suitability of in vivo gold-treated skin as a substrate. Biopsies were obtained from normal-appearing skin of 2 patients with pemphigus with negative AEA titers who had received a cumulative dose of 400 mg or more of GTM. These tissues served as substrates for AEA titer determination on sera of known positivity.

Effect of gold on herpes simplex complement-fixing antibody titer

Herpes simplex complement-fixing antibody titers were assayed using standard techniques [20]. Sera obtained before and during chrysotherapy (500 mg cumulative dose) were simultaneously assayed for herpes simplex complement-fixing antibodies.

Relationship of skin gold concentration to course of pemphigus

Serial skin biopsies (3–10 mg wet weight) were taken from uninvolved skin of 4 patients with pemphigus before and during chrysotherapy using a 4-mm punch. Tissue specimens were placed into acid-washed, preweighed 0.9-ml polyethelene capsules and weighed on a Mettler balance. Capsules, with enclosed specimens, were dried for 6 hr under infrared heat lamps and tissue dry weight measured. Skin gold content was determined by neutron activation. Samples were irradiated at the 0.5 ng sensitivity level. Three blank capsules and 3 ashed fecal control specimens of known value were irradiated to serve as controls.

RESULTS

Effect of gold on prostaglandin synthesis. The oxidation of arachidonic acid by sheep vesicular gland dioxygenase was inhibited by GTM in an instantaneous, concentration-dependent manner (Fig. 1). Addition of soy bean lipoxygenase at the termination of the inhibition experiment resulted in the generation of oxygen; GTM, therefore, did not interfere with the substrates in this reaction, but inhibited the dioxygenase specific for the transformation of arachidonic acid into prostaglandin. Gold, in a final concentration of 49 μM, inhibited 50% of dioxygenase activity, as calculated from a Dixon plot [21]. The inhibitory action of GTM was time-dependent, progressive loss of enzyme activity resulting from longer periods of incubation (Fig. 2). Buffer did not effect the system. In control experiments ^{14}C from arachidonic acid was incorporated into the PGE_2 fraction. Aspirin and indomethacin, known prostaglandin dioxygenase inhibitors [10], produced significant inhibition of activity.

Effect of gold on skin acid phosphatase and tryptophanyl-tRNA synthetase activity. GTM inhibited the activity of human epidermal acid phosphatase; equivalent concentrations of TM did not. The amount of inhibition was proportional to

§ One A_{260} unit of tRNA has an A_{260} of 1.0 in a 1.0-cm optical path when dissolved in 1.0 ml of 5 mM $K_2HPO_{4\cdot5}$ mM K_2HPO_4 at pH 6.9.

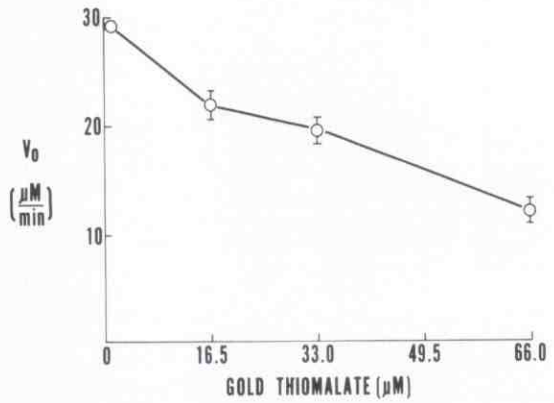


FIG. 1: Concentration-dependent inhibition of vesicular gland dioxygenase activity by GTM. Phenol-activated acetone powder of sheep vesicular gland was added to an assay mixture containing 0.67 M phenol and the indicated concentrations of GTM at 30°C. After 1-min exposure to inhibitor, arachidonic acid (20:4, n-6, final concentration, 20 μM) was added and the reaction rate (μM/min) determined from continuous measurements of oxygen uptake. Each point represents the mean of 5 determinations and is bracketed by ±1 standard deviation.

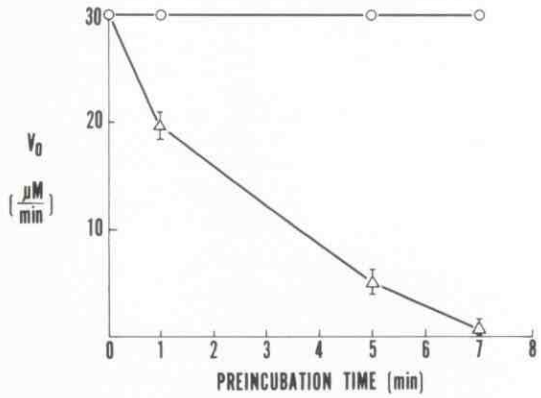


FIG. 2: Time-dependent inhibition of vesicular gland dioxygenase by GTM. Phenol-activated acetone powder of sheep vesicular gland was incubated with 33 μM GTM (△) or no inhibitor (○) for the indicated time periods at 30°C. Arachidonic acid (20:4, n-6, final concentration, 20 μM) was added to the mixture and the reaction rate determined from the initial measurement of oxygen uptake. Each triangle represents the mean of 5 determinations and is bracketed by ±1 standard deviation.

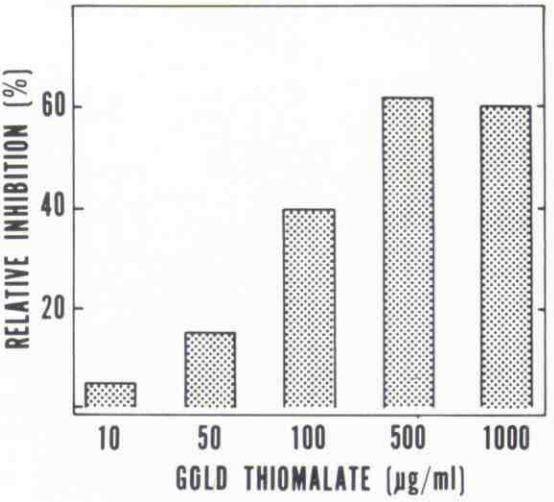


FIG. 3: Inhibition of human epidermal acid phosphatase activity by GTM. Epidermal homogenate was preincubated for 5 min with increasing concentrations of GTM. Acid phosphatase activity was determined as described under *Experimental Procedures*. Relative inhibition is defined as the activity remaining divided by the activity of the untreated control, and is expressed as a percent.

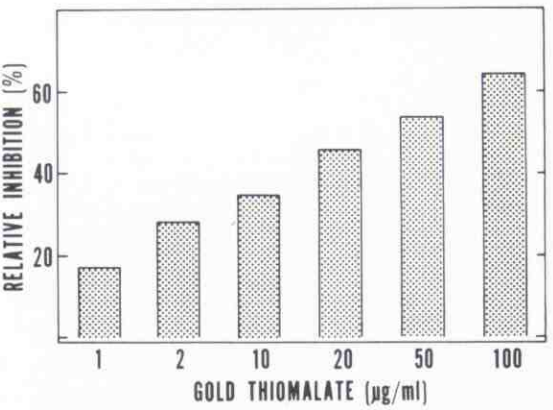


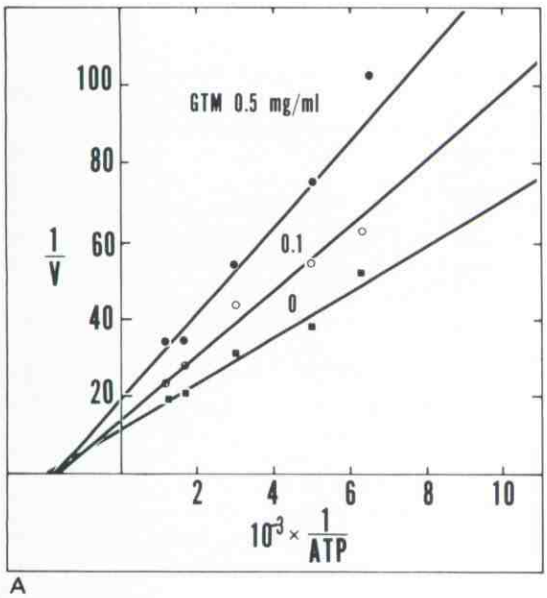
FIG. 4: Inhibition of human epidermal tryptophanyl-tRNA synthetase by GTM. Aliquots of purified tryptophanyl-tRNA synthetase were preincubated for 5 min with increasing concentrations of GTM. Tryptophanyl-tRNA synthetase activity was determined as described under *Experimental Procedures*. Relative inhibition is defined as the activity remaining divided by the activity of the untreated control, and is expressed as a percent.

GTM concentration at concentrations less than 500 μg/ml (Fig. 3).

Similarly, purified human epidermal tryptophanyl-tRNA synthetase was inhibited by GTM (Fig. 4). The degree of inhibition was concentration-dependent and was prevented by 2-mercaptoethanol. Kinetic analysis demonstrated that GTM produced noncompetitive inhibition of all three substrates (ATP, tryptophan, $tRNA^{trp}$) (Fig. 5). Equivalent concentrations of TM had no effect upon enzyme activity.

Effect of GTM on AEA titer. AEA-positive sera were not affected by incubation with GTM, TM, or buffer; similarly, treatment of human skin and monkey esophagus substrates with the above agents did not alter their ability to serve as substrates. Human skin obtained from gold-treated pemphigus patients was also a satisfactory substrate for AEA titer determination.

Effect of gold on herpes simplex complement-fixing antibody titer. Herpes simplex complement-fixing antibody titers did not change as a result of chrysotherapy. There was no evidence of intercurrent herpes infection. Two patients had identical



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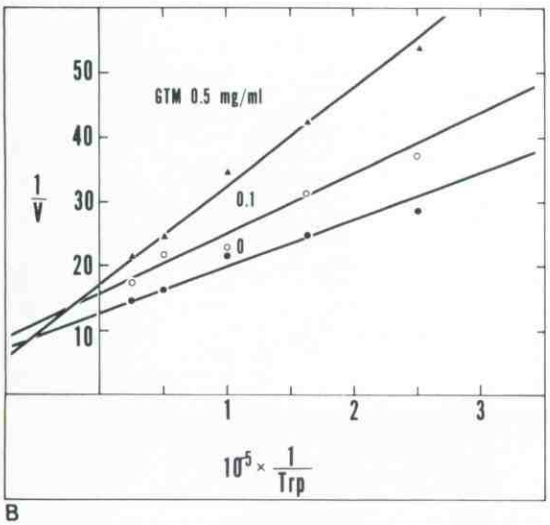

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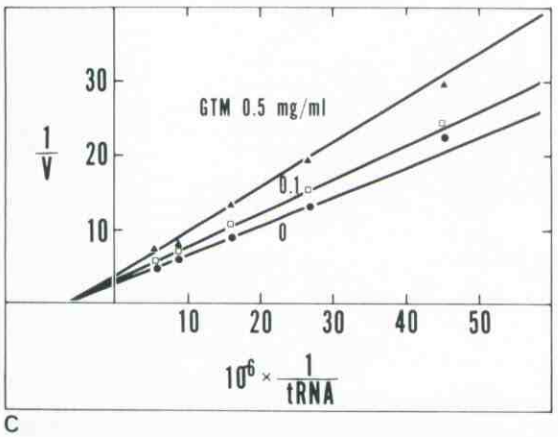

C

FIG. 5: Inhibition of human epidermal tryptophanyl-tRNA synthetase by GTM. The reciprocals of the initial reaction velocities were plotted against the reciprocals of the molar concentrations of L-tryptophan (A), ATP (B), or $tRNA^{trp}$ (C) in the presence and absence of GTM (0.1 or 0.5 mg/ml).

TABLE

Skin gold content as a function of cumulative myochrysine dose

Patient	Skin Au concentration (μg/gm)		Cumulative myochrysine dose (mg)
	Wet weight	Dry weight	
SK	0.2	1.1	10
	0.9	2.6	110
	1.6	5.7	210
	2.8	5.7	360
	2.5	9.0	510
	4.3	14.0	610
EA	0.1	0.3	0
	0.7	1.9	160
	4.1	8.6	460
	7.8	30.6	660
LM	0	0	0
	0.8	1.9	110
	4.2*	13.5	460
	2.6†	10.9	460
TR	0	0	0
	1.5	1.8	110
	3.6	6.9	610

† Skin biopsies taken from skin lesion and uninvolved skin† at the time of lichen planus-like gold dermatitis.

titers (1/8 to 1/8); 1 patient had an increase (1/4 to 1/8), and 1 patient had a decrease of one dilution (1/16 to 1/8).

Relationship of skin gold concentration to the course of pemphigus. Skin gold content increased gradually during gold therapy (Table) [22]. There was no correlation, however, between quantity of gold in skin, cumulative dose of GTM, or activity of pemphigus.

DISCUSSION

The results reported herein demonstrate that GTM affects several biologic systems of possible significance in the pathogenesis or perpetuation of pemphigus. The inhibition of prostaglandin synthesis and human epidermal enzymes in vitro adds two additional, heretofore undescribed, mechanisms of action of gold to the list of its known effects.

Previous studies suggest that certain prostaglandins may be important mediators of inflammation, especially in some forms of arthritis [23, 24] and dermatitis [25]. More recent work suggests that other prostaglandins may also have antiphlogistic properties [26]. While salicylates and indomethacin, two anti-inflammatory agents useful in the management of some arthritic diseases, are potent inhibitors of prostaglandin synthesis, the effect of gold on prostaglandin synthesis was unknown. The results of our studies indicate that GTM at therapeutic serum concentrations [27]

interferes with in vitro prostaglandin synthesis. Skin gold concentrations increased during chrysotherapy, and all patients attained levels of 4 μg elemental gold/gm skin wet weight. This corresponds to an approximate concentration of 20 nmoles/gm of GTM. Assuming that 1 gm wet weight of skin approaches 1 ml, GTM concentration in skin is 20 μM, a level that inhibits prostaglandin synthesis in vitro (Fig. 1). In a concomitant experiment, Ziboh [28] demonstrated that GTM was a more potent inhibitor of prostaglandin synthesis than salicylates, but was less potent than indomethacin. The effect of chrysotherapy on skin prostaglandins in vivo is presently under study.

GTM inhibited two human epidermal enzymes, tryptophanyl-tRNA synthetase and acid phosphatase, in vitro, an effect blocked by 2-mercaptoethanol, a known protector of reduced sulfhydryl groups. Kinetic analysis of the reaction between GTM and tryptophanyl-tRNA synthetase demonstrated noncompetitive inhibition. Saturating quantities of substrate failed to overcome the inhibition, suggesting irreversible complexing between gold and enzyme. The inhibitory effects of gold on selected lysosomal enzymes in human synovial fluid [29] and synovial fluid leukocytes [30] obtained from patients with RA have been reported previously. This is the first time, however, that gold has been shown to inhibit human epidermal enzymes. Thus, gold may interact with enzymes throughout the body, presumably those having a reduced sulfhydryl group available for binding. This hypothesis receives additional indirect support from our earlier work demonstrating measurable quantities of gold in all human tissues that were assayed [31]. Many lysosomal hydrolases are sulfhydryl enzymes [29], and may participate in acantholysis [11]. The beneficial effect of gold in pemphigus may relate, in part, to interference of blister formation by inhibition of degradative lysosomal enzymes such as acid phosphatase.

The binding of AEA to epidermal antigen was not altered by GTM at physiologic or pharmacologic concentrations in vitro. De novo synthesis of antibody, as measured by herpes simplex complement-fixing antibody titer, was not altered by gold administration in vivo. Reduced AEA titers in patients receiving chrysotherapy may be an effect of gold on disease activity with concomitant titer change, rather than a direct effect on AEA. Thus, AEA may be a secondary phenomenon in pemphigus, antibodies forming against released epidermal products from damaged skin. Clinical data suggest that reduction of AEA titers precedes clinical response [11]; this may represent an early response to decreased antigen release.

Attempts to correlate skin gold concentration with the clinical response of pemphigus to chrysotherapy were unrewarding. Gold was measured in whole skin specimens containing unspecified amounts of dermis. Other work [22] has indicated that keratinous tissues have little affinity for gold, in contrast to other heavy metals such as lead and arsenic.

In summary, we have attempted to define the modes of action of gold. In vitro gold in therapeutic serum concentrations has been found to inhibit prostaglandin synthesis and two epidermal enzymes, but had no effect on immunoglobulin synthesis or on the interaction of AEA with epidermal antigen. The results of our study suggest that the final biologic effect of gold in pemphigus may represent a combination of several mechanisms of action, both at a local and systemic level.

Note Added in Proof:

We wish to thank the editor for directing our attention to an article (Deby C, Bacq Z-M, Simon D: In vitro inhibition of the biosynthesis of a prostaglandin by gold and silver. Biochem Pharmacol 22:3141–3243, 1973) that confirms our observation of inhibition of prostaglandin synthesis by gold.

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